Studies on Congenital Hemolytic Syndromes. IV. Gastrointestinal Absorption of Iron

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IN ONLY ONE of the congenital hemolytic syndromes, thalassemia major, is increased storage of iron in tissues consistently pronounced. It may be associated with clinical and pathologic findings similar to those in patients with idiopathic hemochromatosis.[1,2] In thalassemia major, therapy with blood transfusions is regularly required. However, amounts of iron in tissues have been noted to exceed by far those administered in hemoglobin by transfusion.[2] This has indicated that increased gastrointestinal absorption must have been an important source of the storage iron since excretion of this metal is normally minimal.[3-8]

Observations both in animals and in humans have suggested that absorption of iron may be related to presence of anemia, increased erythropoiesis, amount of tissue iron, and level of iron and unbound iron transport protein in the serum (unbound transferrin or latent iron-binding capacity).[9-14] Increased rates of destruction together with increased rates of production of erythrocytes and anemia are common to all congenital hemolytic syndromes, but occur in varying degrees. Results of previous studies have indicated that the following situations may exist in different disorders: markedly increased erythropoiesis and moderately severe anemia (sickle cell anemia); moderately or markedly increased erythropoiesis with mild or absent anemia (sickle cell-hemoglobin C disease, and hereditary spherocytosis); moderately increased effective erythropoiesis with severe anemia (thalassemia major).[15-17,65] In addition, it is known that erythropoiesis can be suppressed and anemia relieved by the administration of transfusions.[18]

The following studies of gastrointestinal absorption of iron were instituted in several congenital hemolytic disorders which might by comparison permit evaluation of possible regulatory factors mentioned above. One patient with congenital "aregenerative" (pure red cell) anemia has been included in order to observe the effect of anemia in the absence of erythropoiesis.

Patient Material

Normal subjects included four well children, aged 6 to 11 years, in whom the hemoglobin concentration was known to be normal for the age at the start of the test period. A second control group consisted of four children, aged 4 to 11 years, who were known

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to be hetrozygous for thalassemia and who were siblings of individuals with homozygous thalassemia. Presence of the heterozygous state was confirmed by finding morphologic abnormalities of erythrocytes and an elevated value for A_2 hemoglobin.[19,20]

In three children, aged 5, 7, and 9 years, with hereditary spherocytosis, the diagnosis was made by presence of spherocytosis and reticulocytosis in the blood of both the patient and of the parent from whom the disorder had been transmitted, as well as by subsequent response to splenectomy.

Sickle cell-hemoglobin C disease was present in two subjects who were mother and daughter. The abnormal hemoglobins were demonstrated by filter paper electrophoresis. Presence of a hemoglobin C trait in the male parent was established by electrophoresis.

Three patients, aged 3, 9, and 12 years, with sickle cell anemia, were shown to be homozygous for sickle cell hemoglobin by electrophoretic studies. Five of the six parents were available for study and were shown to have the sickle cell trait.

Sixteen patients with homozygous thalassemia were studied, of whom six had previously undergone splenectomy. Ages ranged from 5 to 17 years in the non-splenectomized group and 8 to 20 years among the splenectomized patients. The diagnosis had been previously established in each patient by findings of marked anemia, splenomegaly, and bone changes, together with numbers of immature erythrocytes and increased amounts of fetal hemoglobin in the blood, and increased iron in the serum with saturation of the iron-binding protein. The majority of these patients also demonstraed increased pigmentation of the skin. Both parents of each patient had been shown to be heterozygous for thalassemia by measures described above for control subjects.

One patient was included in whom "aregenerative" anemia had been known to be present from the age of three months. The initial diagnosis had been made by the total absence of erythrocyte precursors in the bone marrow in the presence of marked anemia. He had received transfusion therapy since that time. At the age of 7½ years, splenectomy had been performed. The patient was studied at the age of 13; at that time, he required transfusions at intervals of approximately four weeks. A total of 97 transfusions had been given.

Only for patients with thalassemia major and for the single patient with aregenerative anemia was a program of frequent transfusions of sedimented erythrocytes in progress. The least number of transfusions received prior to study in any one of these patients was 28; the greatest was more than 300.

Methods and Calculations

Details of methods for determination of hemoglobin concentration, numbers of erythrocytes, hematocrit, reticulocyte per cent, serum iron and latent iron-binding protein, and filter paper electrophoresis of hemoglobin solutions have previously been described from this laboratory.[15,21]

Rate of reticulocytosis was calculated at the time of study to be:

$$\frac{\text{reticulocyte (\%)} \times \text{number of erythrocytes (millions/cu.mm.) in patient}}{1 \times 5.0 \text{ (millions/cu.mm.) in normal}}$$

$Iron^{59}$ was administered in a dosage of approximately one μc in the presence of five mg. of carrier iron, both in the form of ferrous sulfate. Patients were fasted for 12 hours prior to receiving the medication and for one hour following its administration. Similar aliquots of Fe^{59} were delivered into 100 ml. volumetric flasks in triplicate and diluted with 50 per cent (vol.) HNO_3 for future counting of radioactivity at completion of the study period.

Stool specimens were collected in waxpaper bedpan-liners and transferred (in the waxpaper) into two-quart heat resistant glass jars. In this manner, the entire specimen could be obtained and added to the total collection over a five day period. Patients with thalassemia tended to have numerous, bulky stools, usually two or three per day in number. In the other disorders, number and consistency of stools was normal. Initial

observations in five patients not recorded here failed to reveal detectable radioactivity in specimens collected after five days and usually not after three days following administration of the test dose. After a five day period, the total collection including wax paper was digested by boiling in concentrated sulfuric acid, cooled, homogenized in the same container and the total volume measured. Four ml. aliquots of this mixture were removed in duplicate for counting in a well-type scintillation detector. Four ml. aliquots of the initial or standard specimen diluted 1:100 were also counted at this time. Calculations were as follows:

$$100 \times \frac{\text{specimen counts/min.} \times \text{total volume of specimen in acid}}{\text{Standard counts/min.} \times 100} = \%\ Fe^{59} \text{ in specimen}$$

100 − % in specimen = % of test dose absorbed.

Single observations were obtained in all patients except those with thalassemia major. In the latter group each patient was studied twice: once when the hemoglobin concentration was low, at a time as remote as feasible from the most recent transfusion; and again after the concentration of hemoglobin had been elevated by transfusions of sedimented erythrocytes. At the time of final transfusion prior to the second study, aliquots of the donor erythrocytes labeled with Cr^{51} were injected to permit determination of the survival times of compatibile donor erythrocytes in patients in whom the spleen was *in situ*. This was not done in patients from whom the spleen had been removed because such patients have been shown to demonstrate normal survival of compatible donor erythrocytes for as long as 20 years following splenectomy.[1] This method has been previously recorded.[15] It was shown in patients not recorded here that following oral administration of one μc. of Fe^{59} when the hemoglobin was low and absorption maximal, the orally administered Fe^{59} could not interfere with the interpretation of these Cr^{51} survival studies. Hence, no special discrimination to assure detection of Cr^{51} alone was necessary.

Total erythrocyte volume (Cr^{51}) was determined by the use of erythrocytes taken from the patients' circulation, labeled and reinjected at initiation of both iron studies in splenectomized patients, and at the onset of the test period when the hemoglobin was low in non-splenectomized patients.[15] Total erythrocyte volume in non-splenectomized patients when the hemoglobin had been elevated was assumed to be that determined 2–4 days prior to administration of iron and during institution of the donor erythrocyte survival study by means of a one-hour post-transfusion blood specimen.

In one patient with sickle cell anemia(D. McK.), and in one with sickle cell-hemoglobin C disease (M. McB.), determination of the total erythrocyte volume and of the Cr^{51} survival time of the patient's erythrocytes in his own circulation, as previously described,[15] were performed concurrently with the iron absorption test. In all other patients included in table 2 with sickle cell anemia and hereditary spherocytosis, these studies were done separately but at a time when the value for hematocrit was within 2 per cent of that present during the period of observation of iron absorption except in patient A. D. In this case (hereditary spherocytosis), the hematocrit was 26 per cent at the time of iron administration and 30 per cent at the time of erythrokinetic study.

Results

Four normal children absorbed 5, 8, 17, and 27 per cent and four children with heterozygous thalassemia absorbed 3, 11, 17 and 20 per cent of a test dose of 5 mg. of iron as ferrous sulfate (table 1). In one normal child (patient #4), a low value for serum iron suggested a status of iron deficiency which was not as yet reflected by changes in hemoglobin concentration or erythrocyte number or morphology in the blood. A similar status occurred in one patient (#7) who was heterozygous for thalassemia.

Table 1.—*Absorption of Iron in Control Subjects*

Patient	Age (years)	Hemoglobin (Gm./100 ml.)	Hematocrit (%)	Serum Fe LIBC* (μg./100 ml.)	% A_2† Hemoglobin	Absorption Fe^{59} (%)
			Normal			
1. J. C.	7	13.0	—	98/150	8	27
2. I. K.	6	12.8	41	100/150	–	17
3. D. McC.	11	13.9	43	140/265	–	8
4. M. C.	9	13.0	40	48/260	7	5
			Heterozygous thalassemia			
5. G. LaP.	8	10.2	38	74/260	20	17
6. A. M.	6	10.9	37	135/196	25	20
7. J. T.	4	11.2	34	30/380	15	11
8. J. A. C.	11	10.6	40	130/250	19	3
						range 3–27%
						mean = 13.5%

*LIBC = latent iron-binding capacity.

†Normal = 9.9 ± 3.4 by filter paper electrophoresis.[20]

One patient with aregenerative anemia, or absence of erythropoiesis, absorbed 21 per cent of the test dose of iron (table 2).

In the three patients with hereditary spherocytosis, absorption was 13–23 per cent of the test dose of iron (normal), despite evidence of markedly increased erythropoietic activity (rates of reticulocytosis of 3.9 to 14.5 times the normal rate). Values for hemoglobin concentration at the time of study were 12.7, 10.3 and 8.4 Gm./100 ml. blood (table 2). Similar normal values of 12 and 25 per cent were obtained in two patients with sickle cell-hemoglobin C disease where values for hemoglobin concentration were 12.8 and 13.6 Gm. per 100 ml. blood and rates of reticulocytosis were 3.8 and 2.9 times normal. Serum iron and latent iron-binding capacity tend to be normal in these diseases (see table 5 and Discussion). However, an elevated serum iron with saturation of the iron-binding protein was noted in one patient with hereditary spherocytosis.

In three patients with sickle cell anemia, both marked anemia and active erythropoiesis were present (table 2). Values for hemoglobin concentration were 6.5–8.7 Gm. per 100 ml. blood and rates of reticulocytosis 7.7–8.4 times normal. These patients absorbed 51, 31, and 46 per cent of the test dose of iron, the mean being 42.7 per cent or significantly increased above the control subjects (fig. 1). The value for serum iron was low (46 μg./100 ml.) in one patient (L. A.) with normal total iron-binding protein. Repeat determination of serum iron several months later was 64 μg./100 ml., again with normal amount of total iron-binding protein. In a second patient (D.McK.) the value for serum iron was normal, but the iron-binding protein was saturated. Serum iron determinations in several additional patients with sickle cell anemia are presented in table 5 and will be discussed below.

Results in non-splenectomized patients with severe homozygous thalassemia are presented in table 3. Each patient was studied when the hemoglobin concentration was low and again after hemoglobin concentration had been ele-

vated by transfusion. When values for hemoglobin concentration in these 10 patients were 6.0–8.5 Gm./100 ml. blood at intervals of 7–60 days following the last transfusion, the percentages of iron absorbed were from 12–80 per cent. The mean was 38.9 per cent as compared with a mean of the two control groups of 13.5 per cent. However, it may also be noted that five of these results fall within the range found in the control subjects. Since these patients received frequent transfusions and since suppression of erythropoiesis has been shown to be of variable duration and magnitude, it is not possible to differentiate the effect of the basic disease and the effect of transfusion in producing these results.[18] No correlation could be noted between per cent absorbed and level of hemoglobin concentration, interval from last transfusion, level of serum iron (elevated in all subjects), absence of latent iron-binding capacity (absent in all), presence of splenic effect as evidenced by shortened survival time of donor erythrocytes, number of immature erythrocytes in the peripheral blood, or amount of fetal hemoglobin present. However, when these patients were again studied following elevation of the hemoglobin by transfusion therapy, the following results were obtained: values for hemoglobin concentration were 9.5–12.6 Gm./ml. of blood; in each patient, with two exceptions (patients 2 and 6), the numbers of nucleated erythrocytes in the peripheral blood were smaller than before transfusion, suggesting suppression of erythropoiesis; in each patient, with one exception (patient 2), the amount of iron absorbed was less than that observed during the previous study period. Range of iron absorbed under these circumstances was 0–30 per cent, the mean being 12.6 per cent or essentially the same as that of 13.5 per cent found in the two control groups. In the one patient in whom there was an increase rather than decrease in per cent iron absorbed following transfusion, there was also an increase in number of nucleated erythrocytes in the peripheral blood. Variations in the pattern of the basic disease and short and variable intervals between transfusions make further evaluation of this group not feasible. It may only be stated that following transfusion therapy the amount of iron absorbed was normal. Whether this was the effect of increased hemoglobin concentration or suppression of erythropoiesis could not be determined. It was not associated with any significant consistent changes in levels of serum iron or latent iron-binding capacity.

In the group of patients with severe homozygous thalassemia who had undergone splenectomy more than two years before the test period (table 4), an effort was made to include a rather complete recent transfusion history together with test results. When values for hemoglobin concentration were from 6.2–8.4 Gm./100 ml. blood at intervals of 14–86 days from the last transfusion, the percentages of iron absorbed were 15–78, the mean being 55.5 per cent or significantly increased. When values for hemoglobin concentration were elevated to 9.5–12.5 Gm./100 ml. of blood following transfusion, percentages absorbed were 2–54, the mean being 22.5 per cent. At the time of each test, whether the hemoglobin was high or low, the value for serum iron was markedly elevated and the iron-binding protein completely saturated. Although the result in each patient was lower in the presence of a higher hemoglobin concentration, no direct correlation existed between value

Table 2.—*Hematologic Data and Absorption of Iron in Congenital Hemolytic Syndromes**

Patient	Diagnosis	Age	Hemoglobin (Gm./100 ml.)	Erythrocytes (X 10^6 per cu.mm.)	Hematocrit (%)	Reticulocytes (%)	Serum Fe/LIBC (μg./100 ml.)	Erythrocyte Volume† (ml./Kg.)	Mean Erythrocyte Life† Span (days)	Production of Erythrocytes (rate X normal)	Reticulocytosis (rate X normal)	Absorption of Iron (%)
—	normal	—	14–16	5.0	42–47	0.5–1.5	100/200	29.2	120.0	1.0	1.0	3–27
1. I. Z.	congenital spherocytosis	9	12.7	4.87	35	14.9	—	19.1	20.7	3.7	14.5	16
2. R. B.	congenital spherocytosis	7	10.3	4.10	31	4.8	70/225	20.7	28.6	2.9	3.9	13
3. A. D.	congenital spherocytosis	5	8.4	3.07	26	12.8	196/0	16.6	14.3	4.8	7.9	23
4. M. McB.	sickle-hemoglobin C	34	12.8	4.46	33	3.8	122/200	23.0	35.7	2.7	3.4	12
5. B. McB.	sickle-hemoglobin C	8	13.6	4.56	—	2.9	—	—	—	—	2.6	25
6. D. McK.	sickle cell anemia	3	8.4	2.59	25	14.8	91/0	18.6	13.5	5.7	7.7	31
7. W. A.	sickle cell anemia	9	6.5	2.30	18	18.2	—	18.4	12.0	6.3	8.4	51
8. L. A.	sickle cell anemia	12	8.7	2.98	25	14.0	46/260	17.2	18.0	4.0	8.3	46
9. D. E.‡	aregenerative‡ anemia	13	6.4	2.80	21	0	242/0	9.2	—	0	0	21

*Results in patients with homozygous thalassemia may be found in tables 3 and 4.

†In patients 4 and 6, the Cr^{51} survival studies to determine the mean cell life-span of the patients' own erythrocytes, and the iron absorption studies were performed concurrently. In all patients except #3, the Cr^{51} study was done when the patient was in a stabilized state with hematocrit within 2 per cent of the value recorded at the time of the iron study. In patient #3, hematocrit during the erythrocyte survival study was 30 per cent and reticulocytes were 17.9 per cent.

‡Aregenerative anemia is, of course, not a hemolytic anemia. This patient has been included for the purpose of comparison.

Values for Serum Fe/LIBC (latent iron-binding capacity) were obtained prior to administration of $FeSO_4$.

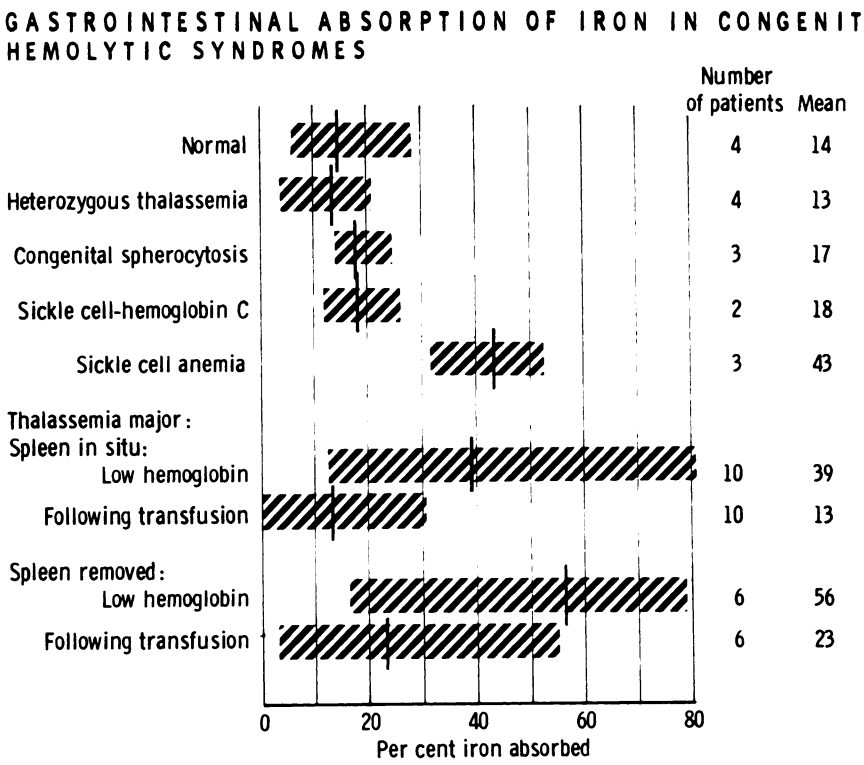

Fig. 1.—Absorption of iron from a test dose of 5 mg. Fe^{++} administered as $FeSO_4$. In only sickle cell anemia and thalassemia major were both marked anemia and increased erythropoiesis present concomitantly. In the latter disease, following elimination of anemia and suppression of erythropoiesis by transfusion, absorption of iron was reduced.

for hemoglobin concentration or red cell volume or increment in either index and per cent iron absorbed. When the results of the two determinations in each patient are analyzed in relation to the number of nucleated erythrocytes per cu.mm. blood (fig. 2), a pattern becomes apparent suggesting a direct correlation of per cent of the test dose of iron absorbed and the number of nucleated erythrocytes in peripheral blood. Results of studies in patient J. N. appeared to vary from those in the other five patients. The only manner in which J. N. was known to differ from the remainder of the group was that he had evidence of early cardiac failure and subsequently died of cardiac complications of this disease. It is, therefore, possible that local hypoxia of intestinal mucosal cells was related to the discrepant results in this patient. The same pattern was present although not so clear when total numbers of immature cells (nucleated erythrocytes plus reticulocytes) were substituted for numbers of nucleated erythrocytes.

Amounts of fetal hemoglobin were decreased in each patient following transfusion therapy, suggesting that suppression of erythropoiesis of some degree was accomplished. However, changes in the amounts could not be directly correlated with changes in iron absorption. This is not unexpected in that

Table 3.—*Hematologic Data and Absorption of Iron in Thalassemia Major—Non-Splenectomized Patients*

Patient	Age	Interval from Recent Transfusions (days)	Hemoglobin (Gm./100 ml.)	Hematocrit (%)	Erythrocytes (X 10^6/cu.mm.)	MCHC*	Fetal Hemoglobin (%)	Serum Fe/LIBC† (μg./100 ml.)	Nucleated Erythrocytes per cu.mm.	Reticulocytes (%)	Erythrocyte Volume (ml./Kg.)	Total Fetal Hemoglobin (Gm./Kg.)	$Cr^{51}T_{½}$ (days) Donor Erythrocytes	Absorption of Iron (%)
Normal			14–16	42–47	5.0	32–36	<2	100/200	0	0.5–1.5	29.2	<0.20	26–32	3–27
1. S. N.	11	A. 35,32,14	8.1	25	3.21	0.32	1.2	–/–	0	<1	17.3	0.07	–	80
		B. 27,20,13,6,3	12.3	37	4.70	0.33	0.2	227/0	0	<1	26.3	0.02	7.0	30
2. S. L.	8	A. 42,28,18,7	6.5	19	2.30	0.34	0.9	155/0‡	48	<1	18.8	0.06	–	13
		B. 27,16,13,9,2	9.5	30	3.14	0.32	0.0	89/135‡	148	<1	33.8	0.00	9.0	17
3. R. C.	9	A. 28,14	8.2	25	2.90	0.33	0.7	164/0	109	<1	23.7	0.06	–	24
		B. 39,21,7,4	12.6	40	4.40	0.32	0.4	147/0	0	<1	31.4	0.04	9.5	3
4. S. I.	6	A. 24,17,14	6.8	21	3.20	0.32	1.4	140/0	360	<1	17.6	0.08	–	12
		B. 27,24,17,7,4	10.7	33	3.78	0.32	0.8	165/0	0	<1	34.0	0.08	12.0	0
5. M. B.	10	A. 28,21	7.5	23	2.91	0.33	0.5	74/0	276	<1	19.8	0.03	–	80
		B. 33,23,19,16,2	12.4	40	4.84	0.31	0.0	51/150	0	<1	32.5	0.00	17.0	12
6. J. M.	9	A. 28,14	6.6	20	2.25	0.33	3.5	161/0	114	<1	19.1	0.21	–	29
		B. 35,21,7,3	12.1	38	4.08	0.32	2.4	–/–	117	<1	30.5	0.24	18.0	18
7. C. C.	10	A. 28,13	6.0	17	2.44	0.35	3.7	208/0	769	<1	15.7	0.20	–	14
		B. 26,14,6,4	10.4	32	3.31	0.32	3.1	227/0	167	<1	31.1	0.31	23.0	9
8. F. DeR.	5	A. 107,105,28	7.5	25	3.20	0.30	21.8	199/0	178	7.4	20.0	1.14	–	76
		B. 42,7,4	12.1	41	4.80	0.30	11.1	165/0	0	<1	28.4	0.94	24.5	5
9. F. P.	17	A. 60	6.0	21	3.10	0.29	14.7	220/0	702	11.2	16.5	0.70	–	42
		B. 52,20,13,4	10.0	32	4.63	0.31	2.3	189/0	56	4.4	31.5	0.22	25.0	21
10. L. C.	7	A. 71,37,34	8.5	22	3.52	0.39	12.2	140/0	597	1.8	15.1	0.72	–	19
		B. 41,6,3	12.3	42	5.29	0.30	7.6	206/0	0	<1	31.6	0.71	32.0	11

*MCHC = mean corpuscular hemoglobin concentration.

†Values obtained prior to administration of $FeSO_4$. LIBC = latent iron-binding capacity.

‡The appearance of measurable amount of LIBC following transfusion in this patient has occurred in repeated determinations.

if this were to be utilized as a true index of erythropoiesis or change in erythropoiesis, it would be necessary to allow sufficient repeated transfusion therapy and time to suppress erythropoiesis and also to permit removal of the patient's fetal hemoglobin-containing erythrocytes from the circulation. Conversely, a sufficient interval would be required between transfusion and study during status of increased erythropoiesis to permit elevation of fetal hemoglobin to its maximal value.

Discussion

Extensive investigations of normal and abnormal absorption and metabolism of iron have produced answers to many questions, but have left many others only partially satisfied. With the advent of subsequent laboratory facilities and technics, numbers of initial impressions have required modification or revision.

In the present studies, $FeSO_4$ containing a tracer amount of $Fe^{59}SO_4$ was employed. Stool analysis for radioactivity was the sole index utilized to measure the amount of iron absorbed from a single test dose of 5 mg. of ferrous iron administered to patients in a fasting state. When evaluating patients in whom variably increased erythrocyte production and destruction are present, this index was thought to offer more accurate results than those obtained by measurement of incorporation of radioiron into erythrocytes.[22] The amount of 5 mg. of elemental iron in the test dose was selected as an approximate representation of the iron content which might be encountered in a single meal. However, the reduced or ferrous form rather than ferric iron as present in food was administered to patients in a fasting state in order that mechanisms of actual absorption of the element might be evaluated when uncomplicated by a number of variable factors which must otherwise be considered.[23-30] Because of the broad range of ages within each test population and the lack of correlation of test results with age and thus with weight in the control groups, it seems unlikely that there has been significant influence by administration of a standard total amount of iron rather than one varied slightly according to body size.[29,31,32]

In this series, values obtained in normal children were comparable to those obtained by other investigators using slightly greater doses of iron but similar methods.[33] Individuals with heterozygous thalassemia may demonstrate a slight anemia, but have not been shown to exhibit hematologic abnormalities other than hypochromia and morphologic aberrations of erythrocytes which survive essentially normally and increased per cent A_2 hemoglobin.[19,20,34,35] Normal absorption of iron from the gastrointestinal tract now becomes an additional feature which distinguishes the heterozygous from the homozygous state in this disorder.

Status of iron stores within the body or bodily need for iron was at one time postulated to be significant in regulation of the amount of iron absorbed.[9,11,14,36] However, the importance of this factor has been decreased by observations in many patients with a variety of disorders (many of them necessitating transfusion therapy) in which absorption of iron has continued in the presence of marked increases in tissue iron. Such observations have

Table 4.—*Hematologic Data and Absorption of Iron in Thalassemia Major—Splenectomized Patients*

Patient	Age	Interval from Recent Transfusions (days)	Hemoglobin (Gm./100 ml.)	Hematocrit (%)	Erythrocytes ($\times 10^6$/cu.mm.)	MCHC*	Fetal Hemoglobin (%)	Serum Fe/LIBC† (μg./100 ml.)	Nucleated Erythrocytes ($\times 10^3$/cu.mm.)	Reticulocytes (%)	Reticulocytes ($\times 10^3$/cu.mm.)	Total Immature Erythrocytes ($\times 10^3$/cu.mm.)	Reticulocytosis (rate X N)	Erythrocyte Volume (ml./Kg.)	Fetal Hemoglobin (Gm./Kg.)	Absorption of Iron (%)
Normal			14–16	42–47	5.0	32–36	<2	100/200	0	0.5–1.5	–	0	1	29.2	<0.20	3–27
1. J. P.	20	A. 86	8.1	30	3.58	0.27	54.2	206/0	45	5.0	180	225	3.6	21.9	3.26	71
		B. 62,16,13,9,2	9.5	35	4.21	0.27	26.0	240/0	11	3.5	147	158	2.9	26.8	1.89	30
2. T. P.	16	A. 86	8.4	32	3.75	0.26	43.2	200/0	53	5.9	221	274	4.4	16.6	1.86	78
		B. 87,16,13,9,2	10.9	39	4.90	0.28	18.9	257/0	8	1.1	54	61	1.1	25.1	1.33	27
3. J. B.	14	A 41	8.1	29	2.99	0.28	4.0	268/0	48	6.2	180	228	3.6	21.2	0.24	48
		B. 59,31,26,6,3	12.5	42	4.14	0.30	2.5	236/0	14	1.6	66	81	1.3	28.6	0.22	2
4. J. LoM.	13	A. 28,14	7.2	23	3.24	0.30	3.6	235/0	29	1.8	58	87	1.2	15.2	0.16	47
		B. 52,22,18,15,3	10.3	33	3.54	0.31	0.0	192/0	6	1.0	35	41	<1	20.2	0.00	15
5. V. P.	8	A. 31	7.8	24	2.82	0.32	2.6	189/0	14	1.5	42	56	<1	15.8	0.13	15
		B. 45,9,6	10.8	36	4.05	0.30	0.9	198/50	0.08	<1	–	–	<1	27.7	0.08	7
6. J. N.	15	A. 45,31,28,14,13	6.2	21	2.52	0.33	1.6	330/0	6	1.0	25	31	<1	14.1	0.07	74
		B. 33,13,6,3	10.5	34	3.84	0.31	0.2	198/0	0.4	<1	15	15	<1	25.8	0.01	54

*MCHC = mean corpuscular hemoglobin concentration.

†Values obtained prior to administration of $FeSO_4$. LIBC = latent iron-binding capacity.

‡It should be noted that nucleated erythrocytes are recorded here as number X 10^3 per cu.mm. Those in table 3 have been expressed as number per cu.mm. Hence these are significantly greater values than those reported for non-splenectomized patients with thalassemia.

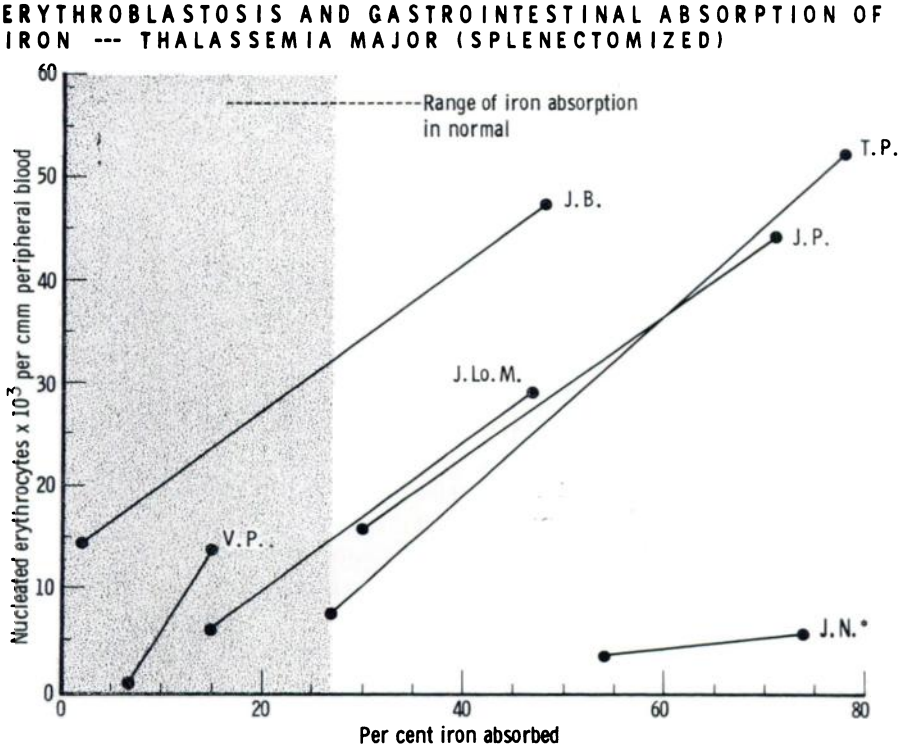

Fig. 2.—Results of two studies in each of six patients with thalassemia major. The first study was performed as long as feasible after transfusion, the second following suppression of erythropoiesis by elevation of hemoglobin concentration by transfusion (point on left). Patient J. N.* was known to be in early cardiac failure and has subsequently succumbed to cardiac complications. Therefore increased anoxia of the intestinal mucosa may have been present.

been accomplished both by measurement of amounts of iron absorbed and by analyses of increased iron content in tissues which could not be accounted for by parenterally administered iron (medication or transfusion).[13,37-48] Continued absorption in the presence of increased stores of iron is again demonstrated by the group of patients with thalassemia major in the present series who were able to absorb amounts of up to 80 per cent of the test dose even in the presence of the iron content of several hundreds of transfusions (tables 3 and 4). Contrariwise, if a reduction from normal of value for serum iron together with an increase in latent iron-binding protein are indeed a reflection of reduced tissue stores of iron, one well child with deficient tissue iron but normal hemoglobin concentration did not absorb an abnormally large amount of iron (table 1, patient 4). Although it is repeatedly stated in the literature that patients with iron deficiency absorb increased amounts of iron, the majority of patients and animals studied have had anemia and, therefore, probably increased erythropoiesis in addition to depletion of tissue stores of iron.[14,30,49,50,67] Perhaps a more accurate statement is to the effect

that patients with iron deficiency *anemia* demonstrate increased absorption of iron.

The level of serum iron has frequently been referred to as a reflection of increased bodily stores of iron. *Available transferrin* or iron-binding protein is necessary to transportation of iron away from the intestinal mucosa. Therefore, the suggestion has been advanced that level of serum iron and degree of saturation of iron-binding protein may regulate iron absorption.[3,12] However, the majority of evidence indicates that increased serum iron and saturation of the iron-binding protein are not in themselves controlling factors to gastrointestinal absorption of iron.[10,14,51-53] In accord with this conclusion, 11 patients with thalassemia major and one with sickle cell anemia absorbed abnormally large amounts of iron despite saturation of the iron-binding protein by chemical determinations.

The significance of serum iron and iron-binding capacity warrants comment. Statements have appeared to the effect that levels of serum iron are increased and amounts of latent iron-binding protein are reduced in the blood of patients with "hemolytic anemias," implying that hemolysis directly affects these values.[54,55] Many such statements appear to result from data from individuals with several disorders in which other factors may be operative.[55] In table 5, values for serum iron and latent iron-binding protein in miscellaneous congenital hemolytic disorders are presented. In all the tabulated congenital hemolytic syndromes except homozygous thalassemia, a normal level of serum iron is common, as is adequate latent iron-binding protein. Patient T. D. with congenital spherocytosis is of particular interest. Serum iron was extremely high and iron-binding protein saturated during an aplastic crisis and subsequently returned to normal following recovery and in the presence of both continued hemolysis and oral iron medication. The aplastic crisis in this patient would be similar to pyridoxine deficiency in which ability to utilize iron is impaired and is associated with elevation of iron and diminution of latent iron-binding protein in serum.[11,56-59] Therefore, saturation of iron-binding protein might occur in any hemolytic disorder as a transitory finding secondary to hypoplasia of the marrow occurring spontaneously or following transfusion. In homozygous thalassemia the iron-binding protein, with few exceptions, is saturated, even in patients with disease of intermediate severity and in the absence of transfusions (tables 3 and 5). Experimental evidence is now accumulating which indicates that a defect is present in homozygous thalassemia in synthesis of heme and, therefore, in utilization of iron.[60-63] The level of serum iron in transfused patients with homozygous thalassemia (tables 3 and 4) is generally higher than with homozygous thalassemia of intermediate severity (table 5). The further increase in the former group might be related either to increased stores of iron from transfusions or to greater severity of the defect in synthesis of heme. The presence of some latent iron-binding protein is to be expected in every individual despite chemical determinations compatible with complete saturation. If this were not so, such a patient might well demonstrate iron toxicity from elements of a normal diet. Figure 3 depicts the electrophoretic pattern of serum from patient J. N. (table 4). Fe^{59} in an amount equivalent to 11 μg. per 100 ml. was added to the serum. The added iron is

Table 5.—*Serum Iron and Latent Iron-Binding Capacity in Congenital Hemolytic Syndromes*

Diagnosis	Patient	Age	Serum Fe/LIBC (μg./100 ml.)	Comment
Congenital spherocytosis	J. M.	8	82/269	
	A. D.	5	196/0	Hgb. 8.4—reticulocytes 13%; previous Hgb. 10.2—reticulocytes 18%.
	R. D.	4	77/110	
	R. B.	7	70/225	
	A. S.	11	75/150	Oral iron medication for one year.
	A. C.	4	112/200	
	L. A.	9	94/100	Oral iron medication for 3½ years.
	B. D.	11	178/175	Recovery phase aplastic crisis and following transfusion.
		12	100/175	
		12	100/240	
	T. D.	8	404/0	Aplastic crisis documented by bone marrow examination. Also transfused from Hgb. 5 Gm. to Hgb. 12 Gm.
		8	225/150	10 days following determination above.
		10	86/165	Hgb. 12. Oral iron medication for 6 years.
		10	132/60	Hgb. 10 Gm.
		10	129/0	3 days following transfusion.
Sickle cell–hemoglobin C	F. B.	4	70/150	
	M. McB.	34	122/200	
Sickle cell–thalassemia	F. P.	14	158/130	No prior transfusions. Clinically well.
	A. V.	14	238/0	Repeated transfusion therapy.
Thalassemia–hemoglobin C	J. M.	10	81/300	
	T. M.	7	90/200	
Sickle cell anemia	D. B.	9	159/100	
	D. McK.	3	91/0	
	L. A.	12	46/260	
		13	64/300	
	S. D.	2	175/250	
		4	95/250	
	G. D.	2	109/150	
	E. C.	7	101/0	4 transfusions in previous year. None within 3 months.
		7	141/0	
	W. C.	2	220/0	
		5	52/220	
		5	76/325	
Homozygous thalassemia (intermediate severity)	C. R.	11	109/0	Hgb. 7.0–9.9 Gm. in absence of transfusions. Fetal hemoglobin 60–82%.
	Z. T.	8	190/0	
	F. S.	10	137/0	
	A. M. S.	3	74/0	
Hemoglobin H	M. J. N.	9	87/120	
	A. Y.	7	140/200	
	N. H.	47	102/200	

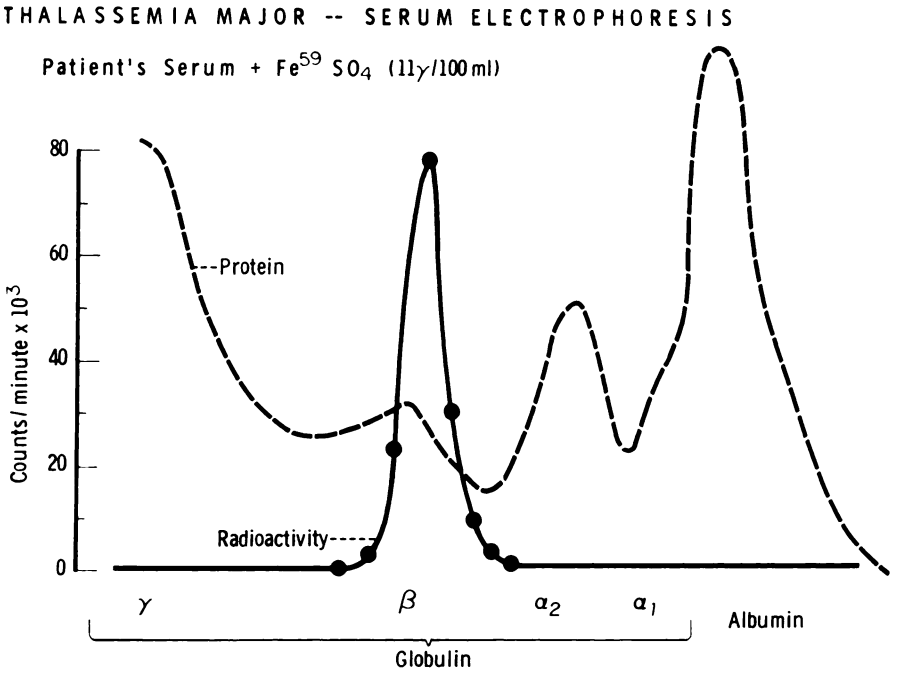

Fig. 3.—Electrophoretic fractionation of serum from J. N. (table 4) to which $Fe^{59}SO_4$ had been added. Peak of radioactivity is present in the area of beta globulin and probably indentifies a small amount of latent iron-binding protein not detectable by chemical determination.

noted to be in the area of the β-globulin and probably identifies a small amount of latent iron-binding protein not detectable by chemical measurement.

The influence of anemia on absorption of iron has been assessed in acute situations in animals where it exerted no immediate effect.[10] Delayed effects may well be attributable to other intermediary forces in association with increased erythropoiesis stimulated by the anemia. In the one patient included in the present study in whom anemia was present in the absence of erythropoiesis (D. E.), no increase in absorption of iron was present. Therefore, anemia *per se* did not produce increased absorption. However, absence of erythropoiesis did not prevent normal absorption.

Increased erythropoiesis present in patients with hereditary spherocytosis and sickle cell-hemoglobin C disease was not associated with an increase in the amount of iron absorbed (figs. 1 and 4). Bothwell has demonstrated absorption of 7.2 ± 2.2 per cent of a test dose of iron of 0.025 mg./Kg. in mice with hereditary spherocytosis, as compared with 4.8 ± 1.9 per cent in control animals.[14] Perhaps expansion of the present series might uncover slight differences with overlap of ranges in normals and in hereditary spherocytosis or perhaps the disease in mice differs from that in humans. From the data presented here, however, it would seem that human patients with hereditary spherocytosis as well as those with sickle cell-hemoglobin C disease with active erythropoiesis and mild anemia do not demonstrate an increased absorption of iron.

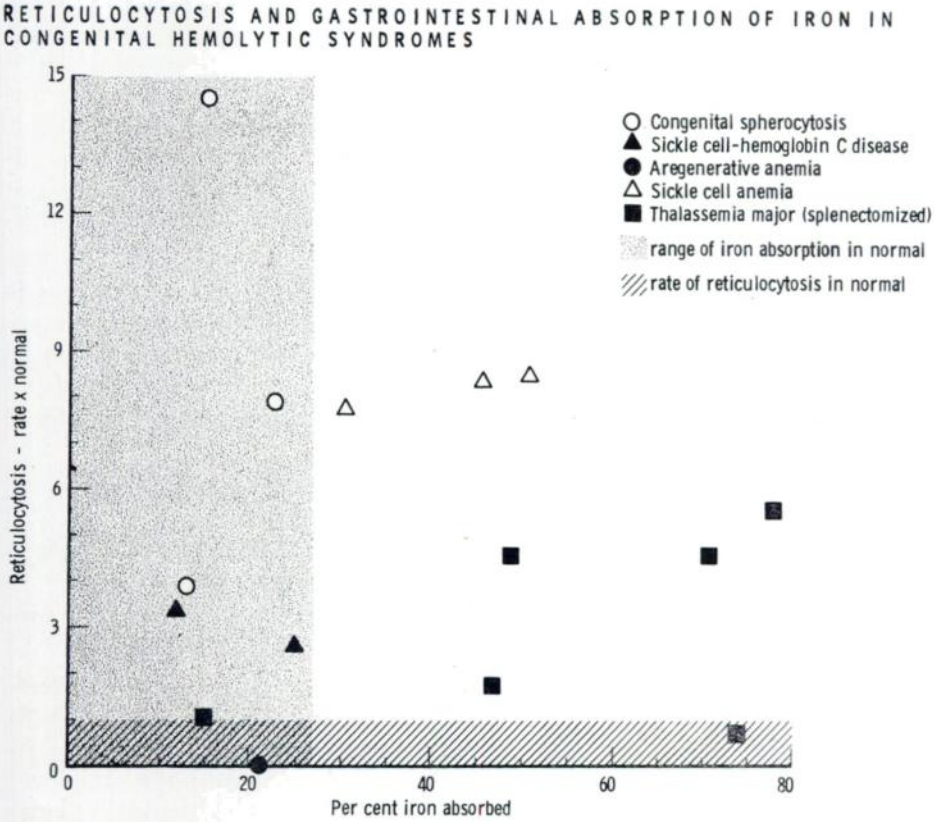

Fig. 4.—Results obtained in patients with thalassemia major are those when the intervals from last transfusion were 14–86 days. Evidence of increased erythropoiesis was present in all subjects except the one patient with aregenerative anemia. However, increased absorption of iron was present only in those with thalassemia major or sickle cell anemia, i.e., in those disorders in which marked anemia coexisted with increased erythropoiesis.

Increased erythropoiesis in patients with sickle cell anemia or homozygous thalassemia was associated with increased absorption of iron. Indeed, if numbers of nucleated erythrocytes in the peripheral blood may be interpreted as a reflection of status of erythropoiesis in splenectomized patients with thalassemia major, the results of studies in these patients are suggestive of a rather direct relationship between erythropoiesis and absorption of iron (fig. 2). Similar investigations before and following transfusions in patients with erythroid hyperplasia, refractory anemia and no increase in hemolysis have been reported to show that ineffective as well as effective erythropoiesis may be associated with increased gastrointestinal absorption of iron which is reduced following transfusions.[64] In thalassemia major a significant amount of ineffective erythropoiesis in addition to effective erythropoiesis has been described.[65,66,68] It is possible that the element of increased ineffective erythropoiesis or initial erythropoietic activity which does not produce viable cells in the peripheral blood accounts for the greater absorption of iron in the presence

of lesser numbers of reticulocytes in patients with thalassemia than in sickle cell anemia with comparable degrees of anemia. (fig. 4).

Comparison of the several diseases studied suggests that in addition to increased erythropoiesis, rather marked anemia must also be present in order for the effect of increased erythropoietic activity on absorption to be manifest. Numbers of patients have been described in the literature who have developed marked siderosis or hemochromatosis following transfusion therapy. In many of these there was a discrepancy between the amount of iron administered by transfusions and that found in tissues, especially liver, postmortem. It is of interest that those patients in whom the amount of iron in tissues was markedly in excess of iron by transfusion have been those patients in whom marked erythroid activity within the marrow had been present.[39-41] This was true also in patients "aplastic" in terms of peripheral blood findings who conform to the picture of refractory anemia with erythroid hyperplasia within the marrow.[40]

Summary

1. Results of studies of gastrointestinal absorption of ferrous iron in normal children and those with heterozygous thalassemia were similar.

2. In one patient with absent erythropoiesis but severe anemia, no increase in the amount of iron absorbed was noted.

3. In sickle cell-hemoglobin C disease and hereditary spherocytosis having only slight anemia in the presence of increased erythropoiesis, normal amounts of iron were absorbed.

4. Patients with sickle cell anemia and thalassemia major in whom there was active erythropoiesis and marked anemia absorbed abnormally large amounts of iron. The amount absorbed by individuals with the latter disease could be reduced by administration of transfusions and concomitant suppression of erythropoiesis.

5. Usual values for serum iron and latent iron-binding capacity in several congenital hemolytic syndromes have been presented and their significance discussed.

6. No specific effect on absorption was noted by increased or reduced amounts of tissue or serum iron or by reduced or increased latent iron-binding protein.

Summario in Interlingua

1. Le resultatos de studios del absorption gastrointestinal de ferro ferrose in juveniles normal e in juveniles con thalassemia heterozygotic esseva simile.

2. In un patiente con absentia del erythropoiese e anemia sever, nulle augmento in le quantitate del ferro absorbite esseva notate.

3. In morbo a cellulas falciforme e hemoglobina C e in spherocytosis hereditari con solmente leve grados de anemia in le presentia de un augmento del erythropoiese, normal quantitates de ferro esseva absorbite.

4. Patientes con anemia a cellulas falciforme e thalassemia major in qui erythropoiese active e grados marcate de anemia esseva presente absorbeva anormalmente grande quantitates de ferro. Le quantitate absorbite per in-

dividuos con le secunde del mentionate morbos poteva esser reducite per le administration de transfusiones e le concomitante suppression del erythropoiese.

5. Es presentate le valores usual pro le ferro seral e pro le latente capacitate ligatori de ferro in le caso de diverse congenite syndromes hemolytic. Le signification del datos es discutite.

6. Esseva notate nulle effecto specific exercite super le absorption de ferro per le augmento o le reduction del ferro de tissu o de sero o per le augmento o le reduction de proteina a latente capacitate ligatori pro ferro.

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